

# The effect of true water hardness and alkalinity on the toxicity of Cu and U to two tropical Australian freshwater organisms

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## 1 Introduction

### 1.1 Background

Metal contamination from mining and industrial activities is an increasing threat to aquatic ecosystems. The presence of metals in the environment increases either directly via atmospheric deposition, wastewater discharge and runoff (eg Pb, Hg, Cd, Cu and Zn), or indirectly as a result of increased solubilisation and mobilisation from sediments (eg Al and Fe). Both marine and freshwater ecosystems are threatened, however, soft freshwaters are particularly sensitive as they are poorly buffered and prone to acidification (McDonald & Wood 1993). In addition, metal speciation and bioavailability in fresh surface waters are known to depend on a variety of physico-chemical parameters (eg temperature, dissolved organic carbon (DOC), pH, hardness and alkalinity). For this reason, the ability to protect aquatic ecosystems from metal contamination depends on understanding tolerance limits of aquatic biota, and the factors influencing these limits. Safe metal concentrations are recommended by the Australian and New Zealand water quality guidelines (ANZECC & ARMCANZ 2001) to protect aquatic ecosystems.

The metals of greatest concern to tropical Australian freshwaters are Al, Cd, Co, Cu, Ni, Mn, Pb, U, V and Zn – largely as a result of mining activities, but also from urban impacts (refer to review by Markich & Camilleri 1997). Copper and U were selected for this study because their toxicity to tropical biota has been comprehensively described. However, high variability in the toxic response of these two metals in tropical freshwaters remains (see Riethmuller (2000), Appendix A). Markich and Camilleri (1997) proposed such variability may be reduced by elucidating the effect physico-chemical parameters (eg hardness, alkalinity, pH, natural organic matter and redox potential) have on the toxicity of these two metals to aquatic biota. Knowledge of the relationship between water chemistry variables, including hardness and alkalinity, and metal toxicity is useful for predicting the potential ecological detriment to aquatic systems, and can be used to modify national water quality guidelines on a site-specific basis.

#### 1.1.1 Significance of water quality guidelines

Water quality guidelines (WQGs) provide a means of assessing the ‘water quality’ required to protect aquatic ecosystems at a prescribed level (Chapman 1995). Currently, Australian and New Zealand aquatic biota are protected by guidelines based predominantly on toxicity data for Northern Hemisphere species (ANZECC & ARMCANZ 2001). However, this has been necessary, as local toxicological data relevant for Australian species are limited or lacking. The ability of Northern Hemisphere data to reflect Australian climatic and limnologic conditions, as well as the phylogeny of species, has often been questioned (Skidmore & Firth 1983, Markich & Camilleri 1997). For example, tropical Australian freshwater systems experience seasonal variations in water quality parameters, such as low conductivity or hardness during the Wet season and high temperature or low dissolved oxygen during the Dry

season, that are beyond ranges so far studied in the Northern Hemisphere (Skidmore & Firth 1983). In addition, the majority of Australia's freshwater fish and invertebrates are endemic, with none of the species used in Northern Hemisphere toxicity tests (predominantly Salmonidae and Cyprinidae) occurring naturally in Australia (Skidmore & Firth 1983). The inability of the previous Australian WQGs (ANZECC 1992) to reflect such environmental differences casts doubt over their level of protection. Of particular concern, is the relevance of temperate-based guidelines for protecting tropical Australian biota, given that tropical Australia encompasses 40% of the Australian continent (ASTECC 1993).

### **1.1.2 Water quality guidelines relevant to tropical Australia**

The main objective of the Australian and New Zealand WQGs is to provide a guide for setting water quality objectives required to sustain current or likely future environmental values for natural and semi-natural water resources (ANZECC & ARMCANZ 2001). The achievement of such an aim is dependent on the quality of toxicological data for Australian and New Zealand biota, and the ability to predict potential impacts on biota under site-specific conditions. The potential impact of mine wastewater and its constituents is just one environmental issue tropical freshwater systems face (Markich & Camilleri 1997). Of particular interest to the present study is the presence of Cu and U in mine wastewaters and the potential environmental impact such metals have on aquatic organisms inhabiting the Wet/Dry tropics. An interim U guideline for Australian freshwaters is  $0.5 \mu\text{g L}^{-1}$  (ANZECC & ARMCANZ 2001). However, there is no provision in the guidelines to use an algorithm to modify the guideline value to account for varying water hardness.

A recent review of available data on metal toxicity to aquatic biota in tropical Australia (Markich & Camilleri 1997) highlighted the need to better describe the tolerance limits of aquatic biota to metals, and the factors influencing these limits. Physico-chemical parameters such as water hardness, alkalinity, pH and dissolved organic carbon (DOC) are factors known to potentially modify metal toxicity and bioavailability (Hamelink et al 1994, Markich et al 2000). Quantitative relationships (algorithms) describing the reduction in bioavailability as a function of increasing water hardness have been established for Cd, Cr(III), Cu, Ni, Pb and Zn. However, water hardness has yet to be quantified and incorporated into the existing water quality guidelines for other metals (eg U). It is recognised that the development of a model based on key water quality variables would enhance the capacity to predict the potential site-specific impacts of U and Cu in tropical aquatic ecosystems.

### **1.1.3 Importance of physico-chemical parameters in determining metal toxicity**

Historically, water quality guidelines have been based on the 'total' aqueous concentration of a metal. However, evidence has established that the 'bioavailable' metal concentration (ie the potential of a metal to enter and interact physiologically with a living system) more accurately predicts the toxic effects of metals (Campbell 1985, Markich 1998). Apart from the potential uptake of the metal, the toxicity of the metal also depends on the form and abundance in which a particular species of metal is present. Metals which are present as the free ion, or as a weak metal complex are more bioavailable than metals in strong complexes or adsorbed to colloidal and/or particulate matter (Markich et al 2000). Physico-chemical variables, such as hardness, pH, alkalinity and DOC may influence the speciation and bioavailability of metals (Hamelink et al 1994, Markich et al 2000). Determining the influence of inorganic complexation is difficult, as the effects of pH, alkalinity and hardness are often difficult to separate. An increase in water hardness is frequently associated with an increase in alkalinity (where Ca and/or Mg are added as carbonate) and often pH. Alkalinity and pH influence metal speciation by changing the free carbonate and hydroxide ion concentration, whereas

hardness (Ca and/or Mg concentration) typically has no direct effect on metal speciation in solution, and only minor indirect effects via changes in ionic strength (Hunt 1987). Calcium and Mg do, however, seem to affect cell membrane permeability and/or compete with trace metals for transport uptake sites (Hunt 1987, Markich & Jeffree 1994).

The Australian and New Zealand water quality guidelines (ANZECC & ARMCANZ 2001) recognises the potential influence of varying hardness on Cd, Cr(III) Cu, Pb, Ni and Zn toxicity in freshwaters. Subsequently, a quantitative method to calculate a metal guideline value with respect to a particular water hardness level has been provided. However, for these guideline values to comprehensively protect aquatic biota, such algorithms need to be derived for other priority metals (eg U).

## 1.2 Copper

### 1.2.1 Significance of Cu in tropical Australian freshwaters

For most aquatic organisms, Cu is essential in trace amounts, but may be one of the most toxic metals when natural concentrations are elevated (Skidmore & Firth 1983, Nor 1987). The revised Australian and New Zealand water quality guidelines for the protection of aquatic ecosystems recommend a Cu concentration between  $0.4 \mu\text{g Cu L}^{-1}$  at  $6 \text{ mg CaCO}_3 \text{ L}^{-1}$  and  $13 \mu\text{g L}^{-1}$  at  $400 \text{ mg CaCO}_3 \text{ L}^{-1}$ , depending on the water hardness (ANZECC & ARMCANZ 2001). The hardness-dependent algorithm used is:

$$\text{HMGV} = 1.4 \left( \frac{\text{H}}{30} \right)^{0.85}$$

where HMGV is the hardness modified guideline value ( $\mu\text{g L}^{-1}$ ) and H is the measured hardness ( $\text{mg CaCO}_3 \text{ L}^{-1}$ ) of a fresh surface water.

### 1.2.2 Chemistry and speciation of Cu in natural waters

In fresh surface waters, Cu may exist in hydrated ionic forms, complexes and/or sorbed to a variety of naturally-occurring organic and inorganic compounds (table 1) (Leckie & Davis 1979, Flemming & Trevors 1989). The free cupric ion ( $\text{Cu}^{2+}$ ) is considered the most predominant form (at  $\text{pH} < 6.5$ ), and the most toxic to aquatic organisms (Markich et al 2000). The concentration of  $\text{Cu}^{2+}$  is controlled by physico-chemical variables including pH, organic ligands/agents (eg humic and fulvic acid) and inorganic ligands (eg phosphates and carbonates) (Markich et al 2000).

**Table 1** Physico-chemical forms of Cu in natural waters<sup>a</sup>

Physico-chemical form	General size and form	Example
Simple ionic species	true solution ( $< 0.001 \mu\text{m}$ )	$\text{Cu}(\text{H}_2\text{O})_6^{2+}$
Weak complexes	"	Cu-fulvic acid
Lipid-soluble complexes	"	Cu-oxinate
Organo-metallic species	"	Cu-citrate
Adsorbed on colloid particles	colloid ( $0.001\text{--}0.1 \mu\text{m}$ )	Cu- $\text{Fe}(\text{OH})_3$ -humic acid
Adsorbed on particles	particulate ( $0.1\text{--}50 \mu\text{m}$ )	Cu adsorbed onto or contained within clay particles

a Modified from Florence and Batley (1980)

The dominance of the free cupric ion ( $\text{Cu}^{2+}$ ) at  $\text{pH} \leq 6.0$  is offset by the abundance of cupric-carbonate and -hydroxy species at  $\text{pH} \geq 7.0$  (Apte & Day 1993). Above  $\text{pH} 6.0$ , the concentration of  $\text{Cu}^{2+}$  declines by an order of magnitude for each 0.5 units increase in pH (Stumm & Morgan 1981). Of the Cu hydroxy species,  $\text{CuOH}^+$  increases in importance over the

pH range 6–8, and  $\text{Cu}(\text{OH})_2$  (aq) increases in importance over the pH range 8–11, while both species are equivalent at approximately pH 8 (Markich et al 2000). The percentage of  $\text{CuCO}_3$  also increases in abundance as the  $\text{Cu}^{2+}$  declines from pH 6 to 8 (Sylva 1976), where the abundance of  $\text{CuCO}_3$  increases with increasing alkalinity, peaking around pH 8 (Miwa et al 1989). The complexation of Cu by sulfate, chloride, nitrate and phosphate depends on the concentration of individual anions, but generally these complexes comprise less than 5% of dissolved Cu in freshwaters (French 1986).

Copper(II) readily complexes with natural dissolved organic matter (DOM), forming strong bonds with ligands containing oxygen, nitrogen and sulfur (Hart 1981, Moore & Ramamoorthy 1984). Subsequently, the majority of Cu in natural waters (90–100%) is present as Cu-DOM complexes, while inorganic Cu species represent a relatively small proportion of the total dissolved Cu (Apte & Day 1993). The percentage of Cu-DOM complexes in freshwaters will increase as the pH and DOM concentration increase, and the concentration of competing ions decrease (Sylva 1976).

The fate of  $\text{Cu}^{2+}$  in aquatic systems is strongly influenced by sorption in the presence of Fe, Al and Mn (oxy)hydroxides, clay and carbonate minerals, insoluble organic matter and biotic surfaces (Leckie & Davis 1979, Dzombak & Morel 1990). Sorption of  $\text{Cu}^{2+}$  to oxyhydroxides increases with pH up to a threshold point, which is dependent on the concentration of Cu, adsorbent, competing ions and ionic strength (Dzombak & Morel 1990). The majority of  $\text{Cu}^{2+}$  is sorbed around pH 7 in most fresh surface waters (Moore & Ramamoorthy 1984, Dzombak & Morel 1990).

### 1.2.3 Toxicity of Cu to tropical Australian freshwater species

The toxicity of Cu to organisms from several phyla, including Chordata (Osteichthyes), Mollusca, Cnidaria, Crustacea and Chlorophyta (see Riethmuller (2000), Appendix A), inhabiting tropical Australian freshwaters has been determined. A recent review by Markich and Camilleri (1997) details this information. Only those studies, that have investigated the toxicity of Cu to Hydra species and purple-spotted gudgeon (*Mogurnda mogurnda*) are discussed here.

#### *Hydra*

The relative sensitivity of freshwater hydra to Cu is difficult to compare (table 2), as authors have used different methodologies. Markich and Camilleri (1997) assessed the toxicity of Cu to the green hydra, *Hydra viridissima*, in a reconstituted soft freshwater (pH  $6.0 \pm 0.1$ , hardness  $3.9 \text{ mg CaCO}_3 \text{ L}^{-1}$ ). Population growth was reduced by 50% (ie effect concentration,  $\text{EC}_{50}$ ) at  $4 \mu\text{g L}^{-1}$  Cu, while the 10% bounded effect concentration ( $\text{BEC}_{10}$ ), an alternative estimate to the no-observed-effect concentration (NOEC), was  $1.6 \mu\text{g L}^{-1}$  Cu (Markich & Camilleri 1997). Pollino and Holdway (1999) found *H. viridissima* to be less sensitive to Cu in a laboratory water at pH  $7.2 \pm 0.4$  (hardness  $20 \text{ mg CaCO}_3 \text{ L}^{-1}$ , and an unknown organic composition). In their study, the 96 h  $\text{LC}_{50}$  (median lethal concentration) and NOEC were calculated to be  $8.5 \pm 0.3$  and  $4.0 \mu\text{g L}^{-1}$  Cu, respectively.

*Hydra viridissima* was found to be three times more sensitive to Cu than the pink hydra, *Hydra vulgaris*, with the 96 h  $\text{LC}_{50}$  values being  $8.5 \mu\text{g L}^{-1}$  and  $26 \mu\text{g L}^{-1}$ , respectively (Pollino & Holdway 1999). Allison and Holdway (1988) also reported *H. viridissima* to be a more sensitive species than *H. vulgaris* to U (table 3). Beach and Pascoe (1998) reported the 48 h and 96 h  $\text{LC}_{50}$  of Cu to *H. vulgaris* to be 190 and  $40 \mu\text{g L}^{-1}$  Cu, respectively, while a 50% reduction in feeding rate was observed at  $10 \mu\text{g L}^{-1}$  Cu. The median lethal concentration may be an important value, however, the substantially lower Cu concentration required to reduce feeding rate, compared with that causing 50% mortality, also has important behavioural implications in assessing environmental impacts.

**Table 2** Summary of Cu toxicity data for Hydra and purple-spotted gudgeon<sup>a</sup>

Species	Water type	pH	Hardness (mg CaCO <sub>3</sub> L <sup>-1</sup> )	Alkalinity (mg CaCO <sub>3</sub> L <sup>-1</sup> )	Test endpoint	Water concentration (µg Cu L <sup>-1</sup> )	Reference
Green hydra ( <i>Hydra littoralis</i> )	Lomis 1954 Synthetic medium	NR <sup>h</sup>	NR <sup>h</sup>	NR <sup>h</sup>	264 h (11 d) mean rate of reproduction	4.0 (LOEC) <sup>f</sup>	Stebbing & Pomroy (1978)
Green hydra ( <i>Hydra viridissima</i> )	Synthetic Magela Creek	6.0 ± 0.1	3.9 (3.8–4.0)	4.1 (4.0–4.2)	96 h population growth	1.6 (BEC <sub>10</sub> ) <sup>b</sup>	Markich & Camilleri (1997)
						4.0 (MDEC) <sup>c</sup> (EC <sub>50</sub> ) <sup>d</sup> (3.8–4.2)	
Pink hydra ( <i>Hydra vulgaris</i> )	Autoclaved mains	7.2 ± 0.4	20	NR <sup>h</sup>	96 h population growth	4 (NOEC) <sup>e</sup>	Pollino & Holdway (1999)
						(LOEC) <sup>f</sup> (LC <sub>50</sub> ) <sup>g</sup> 8 8.5	
Purple-spotted gudgeon ( <i>Mogurnda mogurnda</i> )	Autoclaved mains	7.2 ± 0.4	20	NR <sup>h</sup>	96 h population growth	4 (NOEC) <sup>e</sup>	Pollino & Holdway (1999)
						(LOEC) <sup>f</sup> (LC <sub>50</sub> ) <sup>g</sup> 8 26	
Purple-spotted gudgeon ( <i>Mogurnda mogurnda</i> )	Buffalo Billabong	6.5	4 (3–5)	3 (2–4)	96 h sac-fry survival	20 (NOEC) <sup>e</sup>	Rippon & Hyne (1992)
						(LOEC) <sup>f</sup> > 200 (LOEC) <sup>f</sup>	
Purple-spotted gudgeon ( <i>Mogurnda mogurnda</i> )	Synthetic Magela Creek	6.0 ± 0.1	3.9 (3.8–4.0)	4.1 (4.0–4.2)	120 h embryo hatching		
					96 h survival	12 (BEC <sub>10</sub> ) <sup>b</sup>	13
						(MDEC) <sup>c</sup> (LC <sub>50</sub> ) <sup>g</sup> (22–24)	

<sup>a</sup> All numerical values represent mean values, or their range, with 95% confidence intervals (C.I) in parentheses (where reported). Means shown with ± values were regulated within the reported limits.

<sup>b</sup> BEC<sub>10</sub>, 10% bounded-effect concentration (Hoekstra & van Ewijk 1993), an analogous statistical measure of the no-observed effect concentration (NOEC)

<sup>c</sup> MDEC, minimal detectable effect concentration (Ahsanullah & Williams 1991), an analogous statistical measure of the lowest-observed effect concentration (LOEC)

<sup>d</sup> EC<sub>50</sub>, median effect concentration

<sup>e</sup> NOEC, no-observed effect concentration

<sup>f</sup> LOEC, lowest-observed effect concentration

<sup>g</sup> LC<sub>50</sub>, concentration at which there is 50% survival

<sup>h</sup> Not reported

Stebbing and Pomroy (1978) investigated the response of a temperate hydra species, *Hydra littoralis*, to Cu. The rate of asexual reproduction was significantly ( $P \leq 0.05$ ) inhibited by  $4.0 \mu\text{g L}^{-1}$  Cu. It is difficult to compare the response of *H. littoralis* to the tropical species *H. viridissima* and *H. vulgaris*, due to differences in experimental conditions such as test endpoint and physico-chemical parameters of the test waters (table 2). Stebbing and Pomroy (1978) reported a linear relationship between metal levels accumulated in hydra tissue and nominal metal exposure levels. This supports the finding that *Hydra* sp, like other aquatic invertebrates, are unable to regulate the uptake of Cu (Bryan 1976, Hyne et al 1993).

#### *Purple-spotted gudgeon (M. mogurnda)*

The sensitivity of *M. mogurnda* to Cu appears to differ between natural water and synthetic water (inorganic component of natural water) (table 2). This is not surprising considering Cu toxicity is known to decrease in the presence of organic matter (Breault et al 1996). In natural Magela Creek water (Buffalo Billabong), Rippon and Hyne (1992) found a 96 h NOEC of  $20 \mu\text{g L}^{-1}$  Cu and a 96 h lowest observed effect concentration (LOEC) of  $64 \mu\text{g L}^{-1}$  Cu on *M. mogurnda* sac-fry survival. Markich and Camilleri (1997) reported *M. mogurnda* to be two-fold more sensitive in synthetic water than natural water, having a  $\text{BEC}_{10}$  of  $12 \mu\text{g L}^{-1}$  and a minimum detectable effect concentration (MDEC, analogous to LOEC) of  $13 \mu\text{g L}^{-1}$ . Such a difference between studies could be explained by the reduction in bioavailable Cu concentration in the natural water as a result of Cu-organic complexation. However, Rippon and Hyne (1992) did not measure the dissolved organic carbon (DOC) concentration in their test water. The sensitivity of *M. mogurnda* to Cu has not been directly compared with a range of other species in a single study, however, *M. mogurnda* appears to be among the more sensitive fish species to metals compared with those investigated in independent studies (see Riethmuller (2000), Appendix A). This supports the findings of Bywater et al (1991) for U sensitivity.

#### **1.2.4 Mechanisms of Cu toxicity in water**

Upon diffusion of a metal through the protective layer of a living organism, the incoming metal will encounter a range of potential binding sites (Campbell 1995). The metal may 'collect' without affecting normal cell function or be taken up, perturbing processes such as photosynthesis, respiration, motility, growth and reproduction.

Copper is considered highly toxic to hydra, particularly green hydra (*H. viridissima*). The symbiotic algae hosted by *H. viridissima* help regulate exposure to elevated levels of a metal by accumulating excess metal and being shed from the host tissue (Hyne et al 1993). However, this mechanism may be inadequate in the presence of Cu due to Cu being such a potent algicide (Pollino & Holdway 1999). Copper has been found to inhibit the photosynthesis of an Australian tropical *Chlorella* sp at  $1.6 \mu\text{g L}^{-1}$  Cu (Franklin et al 1998). At non-toxic concentrations, Cu has been reported to increase hydra population growth (Stebbing & Pomroy 1978, Pollino & Holdway 1999). Hormesis was found to occur at Cu concentrations below  $5 \mu\text{g L}^{-1}$  for *H. littoralis* (Stebbing & Pomroy 1978) and below  $8 \mu\text{g L}^{-1}$  for *H. vulgaris* and *H. viridissima* (Pollino & Holdway 1999).

Gill surfaces of fish have been identified as the primary uptake site of several waterborne metals ( $\text{Cu}^{2+}$ , Laurén & McDonald 1986, Reid & McDonald 1991,  $\text{Cd}^{2+}$ , Part et al 1985, Reid & McDonald 1988,  $\text{Zn}^{2+}$ , Hogstrand et al 1994,  $\text{Al}^{3+}$ , Verbost et al 1992). The permeability of the gill surface is expected to be greater if the membrane has a low affinity for the metal (Reid & McDonald 1991). Once through the membrane and in the intracellular compartment the metal is exposed to various complexing ligands. Metals may bind to these ligands, resulting in one or more of the following mechanisms of toxicity: a) blocking of essential biological functional groups in biomolecules; b) displacement of essential metal ions in molecules; and

c) modification of active conformation of biomolecules (Reid & McDonald 1991). These mechanisms can be used to describe osmoregulation inhibition by  $\text{Cu}^{2+}$  exposure (Laurén & McDonald 1986, Reid & McDonald 1988). Copper(II) has been found to disrupt gill functioning by forming covalent bonds with nitrogen/sulphur-rich ligands such as those of APTase (Reid & McDonald 1988).

Surface bound  $\text{Ca}^{2+}$  is known to stabilise the gill membrane, consequently reducing ionic permeability (Flik & Verboost 1994). It is hypothesised that increased  $\text{Ca}^{2+}$  concentrations in solution further protect aquatic biota from toxic trace metals by competing with the free ionic species for binding sites at the gill surface (Markich & Jeffree 1994).

The  $\text{H}^+$  ion has been found to disrupt gill functioning in rainbow trout (*Salmo gairdneri*, renamed *Oncorhynchus mykiss*) by impairing transepithelial ion exchange (Reid & McDonald 1988). The mechanism by which  $\text{H}^+$  affects gill permeability may be related to its charge, ionic radius, ligand binding preference (eg oxygen versus nitrogen or sulphur ligands) and binding affinity (Reid & McDonald 1991).

### **1.2.5 Effect of physico-chemical parameters on Cu toxicity**

It is generally accepted that increased water hardness reduces the toxicity of Cu to freshwater organisms (see reviews by Sorensen 1991 and Mayer et al 1994). Conversely, Winner (1985) and Laurén and McDonald (1986) found increasing hardness had little or no effect on the toxicity of Cu. Several studies (Howarth & Sprague 1978, Gauss et al 1985, Belanger et al 1989) that provide evidence in support of the inverse relationship between hardness and Cu toxicity confounded the effects of true water hardness (ie Ca and/or Mg concentrations) by accompanying changes in hardness with changes in alkalinity and pH. For example, Howarth and Sprague (1978) reported the 96 h  $\text{LC}_{50}$ , for rainbow trout (*Salmo gairdneri*) exposed to Cu, to vary from 20  $\mu\text{g L}^{-1}$  in soft acid water, to 520  $\mu\text{g L}^{-1}$  in hard alkaline water, where hardness ranged from 30 to 360  $\text{mg CaCO}_3 \text{ L}^{-1}$ , and pH from 5 to 9. In many freshwater systems hardness has a strong positive correlation with alkalinity and pH, however, confounding the effects of these physico-chemical parameters has important implications if the effects of 'true water hardness' on Cu toxicity are assumed to be constant over an infinitely wide range of water qualities.

Several studies have identified the need to discern the effects of true water hardness (ie Ca and/or Mg concentration) on copper-organism interactions, and have successfully described the relationship by maintaining constant alkalinity and pH. Increasing water hardness was found to ameliorate the toxicity and bioavailability of Cu to aquatic biota (Miller & Mackay 1980, Mierle 1981, Horne & Dunson 1995 and Erickson et al 1996). The toxicity of Cu is reduced by the  $\text{Ca}^{2+}$  and/or  $\text{Mg}^{2+}$  ions competing with  $\text{Cu}^{2+}$  ions for binding sites at the cell surface of organisms, without directly affecting Cu speciation (Markich & Jeffree 1994, Erickson et al 1996). More specifically, Ca has been identified as having a greater inhibitory effect than Mg on the toxicity of Cu to aquatic organisms (O'Shea & Mancy 1978, Erickson et al 1996).

The effect of increasing alkalinity ( $\log \text{pCO}_2$ ) has also been reported to reduce the toxicity and bioavailability of Cu to freshwater biota, in experiments that manipulated the carbonate concentration independently of the Ca and Mg concentration, and pH (Andrew et al 1977, Miller & Mackay 1980, Laurén & McDonald 1986, Daly et al 1990b). Alkalinity may have reduced Cu toxicity via the formation of Cu-carbonate complexes, which decrease the activity of the free metal ion ( $\text{Cu}^{2+}$ ) (Borgmann 1983, Hunt 1987).

Solution pH is a primary variable influencing the toxicity of metals, yet the literature describes opposing effects of pH. Many studies have found Cu to be less toxic with freshwater acidification, over a pH range of 3.0 to 7.0 (Campbell & Stokes 1985, Cusimano et al 1986, Macfie et al 1994, Horne & Dunson 1995, Franklin et al 1998). The protective effect of low pH on Cu toxicity is considered a function of H<sup>+</sup> competitively inhibiting Cu<sup>2+</sup> at metal transport sites on the cell membrane (Pagenkopf 1983, Campbell & Stokes 1985, Gerhardt 1993). In contrast, some studies have reported an increase in Cu toxicity with a reduction in pH, over the pH range 6.0 to 8.5 (Waiwood & Beamish 1978, Schubauer-Berigan et al 1993, Erickson et al 1996).

Copper toxicity has been reported to decrease in the presence of organic complexing agents (Meador 1991, Welsh et al 1993, Azenha et al 1995, Erickson et al 1996, Hansten et al 1996); other studies suggest that under certain conditions Cu toxicity may be enhanced (Guy & Kean 1980, Daly et al 1990a, Tubbing et al 1994, Buchwalter et al 1996). The attenuating effects of natural DOC (eg humic and fulvic acids) and synthetic organic agents (eg EDTA) in surface waters (pH 5–9) are attributed to their ability to complex with Cu. In contrast, Cu-organic complexes may increase Cu toxicity by facilitating the transport of cupric ions into cells and/or increasing cell permeability (Guy & Kean 1980, Daly et al 1990a). Further studies are required to validate such relationships.

## 1.3 Uranium

### 1.3.1 Significance of U in tropical Australian freshwaters

The surface waters of rivers and streams in tropical Australia, particularly the Northern Territory, typically contain less than 1 µg L<sup>-1</sup> U (Hart et al 1987, Markich 1998). Uranium is non-essential for biological processes and is generally toxic at elevated concentrations (Berlin & Rudell 1979). Since U is highly soluble and mobile in natural waters (Morse & Choppin 1991), contaminated waters from local mining activities are a potential hazard to aquatic biota. Given the presence of U mines in tropical Australia, the toxicity of U to freshwater biota has been frequently studied (Bywater et al 1991, Holdway 1992, Markich & Camilleri 1997, Markich 1998).

### 1.3.2 Chemistry and speciation of U in natural waters

In aquatic environments, U may exist in many soluble forms, including the dissolved uranyl ion (UO<sub>2</sub><sup>2+</sup>) and other uranyl complexes such as (UO<sub>2</sub>)<sub>3</sub>(OH)<sub>5</sub><sup>+</sup>, UO<sub>2</sub>(CO<sub>3</sub>)<sub>2</sub><sup>2-</sup> and (UO<sub>2</sub>(HPO<sub>4</sub>)<sub>2</sub>)<sup>2-</sup> (Langmuir 1978, Markich et al 1996). There is considerable evidence suggesting the hexavalent uranyl ion (UO<sub>2</sub><sup>2+</sup>) predominates in oxidised surface waters and forms stable, readily soluble, cationic, anionic and/or neutral complexes that are highly mobile (Langmuir 1978, Osmond & Ivanovich 1992, Markich et al 1996). Suspended particles, pH, redox potential, organic complexes and inorganic ligands (such as phosphates and carbonates) govern the speciation of U and its abundance in natural waters.

The speciation of U is highly pH-dependent. At pH ≤5.0, the free hydrated uranyl ion (UO<sub>2</sub><sup>2+</sup>) predominates, becoming insignificant at pH ≥6.0, in waters containing environmentally relevant concentrations of dissolved U (<10 µg L<sup>-1</sup>) (Grenthe et al 1992, Markich et al 1996). The second most dominant species at pH 5 is UO<sub>2</sub>OH<sup>+</sup>, which increases in importance up to pH 6 (Grenthe et al 1992, Markich et al 1996). The formation of polymeric uranyl-hydroxide complexes including (UO<sub>2</sub>)<sub>2</sub>(OH)<sub>2</sub><sup>2+</sup>, (UO<sub>2</sub>)<sub>3</sub>(OH)<sub>5</sub><sup>+</sup>, (UO<sub>2</sub>)<sub>4</sub>(OH)<sub>7</sub><sup>+</sup> and (UO<sub>2</sub>)<sub>3</sub>(OH)<sub>7</sub><sup>-</sup> increase in importance at pH ≥5.0, particularly at higher U concentrations (Grenthe et al 1992, Markich et al 1996). Markich et al (2000) provided evidence to suggest that UO<sub>2</sub><sup>2+</sup> and, to a

lesser extent,  $\text{UO}_2\text{OH}^+$  are the U species that contribute most to the toxic response observed in aquatic biota, where  $\text{UO}_2^{2+}$  has approximately twice the effect of  $\text{UO}_2\text{OH}^+$ .

Carbonate is considered the most significant inorganic complexing agent of uranyl ions due to the formation of very stable complexes (Greene et al 1986). In moderate to hard waters (ie hardness and alkalinity  $>60 \text{ mg CaCO}_3 \text{ L}^{-1}$ ) at pH 5–6,  $\text{UO}_2\text{CO}_3$  is the dominant species, while at pH 6–8,  $\text{UO}_2(\text{CO}_3)_3^{4-}$  is the dominant species. The complexation of uranyl by chloride, sulfate, nitrate and silicate is considered relatively weak compared with uranyl complexes with carbonate and phosphate in freshwaters (Gascoyne 1992). Uranyl-phosphate complexes only start to become significant when the concentration of phosphate approaches  $100 \mu\text{g/L}$  (Langmuir 1978).

Dissolved organic matter, as humic and fulvic acids, is known to form stable complexes with uranyl ions in natural waters (Choppin 1992, Markich 1998). Soluble uranyl-DOM complexes contribute to the migration of uranyl ions in water (Moulin et al 1992), while insoluble uranyl-DOM complexes may reduce the bioavailability and toxicity of U to aquatic organisms by acting as a sink for U (Brown et al 1994). In organic-rich freshwaters that have a low hardness and alkalinity (pH 5–7), the uranyl-DOM complexes are considered the dominant species of dissolved U (Markich 1998). However, as the hardness, alkalinity and pH (usually pH  $>7$ –8) of the water increases, there is a shift in speciation where uranyl-carbonate and uranyl-hydroxide-carbonate species become more important than uranyl-DOM complexes (Moulin et al 1992).

The fate of U in freshwaters is also known to be significantly influenced by sorption to clay minerals below pH 5, and Fe and Al (oxy)hydroxides, silica and microorganisms at higher pH (Greene et al 1986, McKinley et al 1995, Kohler et al 1996). Sorption of U to particles is typically elevated with increasing pH up to a threshold point, which depends on the concentration of U, adsorbent, competing ions (eg carbonate), chelating agents and ionic strength (Markich et al 2000). In fresh surface waters (pH 6–8), the solubility of uranyl minerals is close to minimum (Langmuir 1978), while the sorption of uranyl by organic matter is close to maximum (Choppin 1992).

### 1.3.3 Toxicity of U to tropical Australian freshwater species

The toxicity of U to organisms from several phyla, including Chordata (Osteichthyes), Mollusca, Cnidaria, Crustacea and Chlorophyta (see Riethmuller (2000), Appendix A), inhabiting tropical Australian freshwaters has been determined. This database contains an extensive summary of U ecotoxicological information, which is non-existent for other tropical continents. A recent review by Markich and Camilleri (1997) detailed this information. Only those studies that have investigated the toxicity of U to hydra species and purple-spotted gudgeon (*M. mogurnda*) are discussed here.

#### *Hydra*

The toxicity of U to hydra has been reported in three studies (table 3). Allison and Holdway (1988) investigated the effects of U toxicity to population growth of green hydra (*H. viridissima*) and pink hydra (*H. vulgaris*). *H. viridissima* was found to be approximately 5-fold more sensitive to U than the pink hydra, *H. vulgaris* (table 3). Investigations using natural Magela Creek (Buffalo Billabong) water found U concentrations  $\geq 160 \mu\text{g L}^{-1}$  inhibit *H. viridissima* population growth (Allison & Holdway 1988). However, in synthetic Magela Creek water, *H. viridissima* was three times more sensitive to U with an  $\text{EC}_{50}$  of  $108 \mu\text{g L}^{-1}$  (Markich & Camilleri 1997). Despite the ionic composition of the synthetic water mimicking the natural creek water, the slightly greater pH ( $\sim 0.5$  units) and un-reported DOC content of the natural water probably contribute to the apparent difference.

**Table 3** Summary of U toxicity data for hydra species and purple-spotted gudgeon<sup>a</sup>

Species	Water type	pH	Hardness (mg CaCO <sub>3</sub> L <sup>-1</sup> )	Alkalinity (mg CaCO <sub>3</sub> L <sup>-1</sup> )	Test endpoint	Water concentration (µg U L <sup>-1</sup> )	Reference
Green hydra ( <i>Hydra viridissima</i> )	Buffalo Billabong	6.5 ± 0.2	4 (3–5)	3 (2–4)	96 h population growth	160 (LOEC) <sup>f</sup> (Dry season)	Allison & Holdway (1988)
						194 (LOEC) <sup>f</sup> (Wet season)	
Pink hydra ( <i>Hydra vulgaris</i> )	Synthetic Magela Creek	6.0 ± 0.1	3.9 (3.8–4.0)	4.1 (4.0–4.2)	96 h population growth	56 (BEC <sub>10</sub> ) <sup>b</sup> 61 (MDEC) <sup>b</sup> 108 (EC <sub>50</sub> ) <sup>b</sup> (102–114)	Markich & Camilleri (1997)
						740 (LOEC) <sup>f</sup> (Dry season)	
Purple-spotted gudgeon ( <i>Mogurnda mogurnda</i> )	Buffalo Billabong	6.4 ± 0.1	4 (3–5)	3 (2–4)	96 h population growth	2340 (LC <sub>50</sub> ) <sup>g</sup> (1860–2790)	Allison & Holdway (1988)
						1265 (LC <sub>50</sub> ) <sup>g</sup> (950–1650)	
Purple-spotted gudgeon ( <i>Mogurnda mogurnda</i> )	Magela Creek	6.6 ± 0.1	4.8 (4.6–5.0)	3.3 (3.0–3.6)	48 h survival	1265 (LC <sub>50</sub> ) <sup>g</sup> (950–1650)	Bywater et al (1991)
						2450 (LC <sub>50</sub> ) <sup>g</sup> (1960–2990)	
Purple-spotted gudgeon ( <i>Mogurnda mogurnda</i> )	Magela Creek	6.6 ± 0.1	4.8 (4.6–5.0)	3.3 (3.0–3.6)	72 h survival	1665 (LC <sub>50</sub> ) <sup>g</sup> (1280–2170)	Bywater et al (1991)
						1665 (LC <sub>50</sub> ) <sup>g</sup> (1280–2170)	
Purple-spotted gudgeon ( <i>Mogurnda mogurnda</i> )	Buffalo Billabong	6.4 ± 0.1	3.2 (3.0–3.4)	3 (2.8–3.2)	336 h (14 d) survival	1000 (NOEC) <sup>e</sup> 2040 (LOEC) <sup>f</sup>	Holdway (1992)
						502 (NOEC) <sup>e</sup> 1000 (LOEC) <sup>f</sup>	

**Table 3** Cont'd

Species	Water type	pH	Hardness (mg CaCO <sub>3</sub> L <sup>-1</sup> )	Alkalinity (mg CaCO <sub>3</sub> L <sup>-1</sup> )	Test endpoint	Water concentration (µg U L <sup>-1</sup> )	Reference
Purple-spotted gudgeon ( <i>Mogurnda mogurnda</i> )	Buffalo Billabong	6.3 ± 0.2	4.1 (4.0–4.2)	1.8 (1.7–1.9)	168 h (7 d) survival	1810 (LC <sub>50</sub> ) <sup>g</sup> (1730–1780)	Holdway (1992)
					168 h (+ 168 h post exposure)	1015 (LC <sub>50</sub> ) <sup>g</sup> (900–1190)	
					168 h (7 d) growth	920 (NOEC) <sup>e</sup> 1780 (LOEC) <sup>f</sup>	
					168 h (+ 168 h post exposure)	<455 (NOEC) <sup>e</sup> 455 (LOEC) <sup>f</sup>	
					96 h survival	1790 (LC <sub>50</sub> ) <sup>g</sup> (1385–2100)	
					96 h growth	640 (NOEC) <sup>e</sup> 1240 (LOEC) <sup>f</sup>	
Purple-spotted gudgeon ( <i>Mogurnda mogurnda</i> )	Buffalo Billabong	6.3 ± 0.2	5.1	3.2	96 h survival	3750 (LC <sub>50</sub> ) <sup>g</sup> (2580–4925)	Holdway (1992)
					168 h (7 d) survival	3070 (LC <sub>50</sub> ) <sup>g</sup> (2580–3590)	
					168 (+168 h post exposure)	1640 (LC <sub>50</sub> ) <sup>g</sup> (1120–2565)	
					168 h growth	2580 (NOEC) <sup>e</sup> 4930 (LOEC) <sup>f</sup>	
					168 h (+ 168 h post exposure)	1240 (NOEC) <sup>e</sup> 2580 (LOEC) <sup>f</sup>	

**Table 3** Cont'd

Species	Water type	pH	Hardness (mg CaCO <sub>3</sub> L <sup>-1</sup> )	Alkalinity (mg CaCO <sub>3</sub> L <sup>-1</sup> )	Test endpoint	Water concentration (µg U L <sup>-1</sup> )	Reference
Purple-spotted gudgeon ( <i>Mogurnda mogurnda</i> )	Buffalo Billabong	6.6 ± 0.2	5.1	3.2	96 h survival  168 h (7 d) survival	3750 (LC <sub>50</sub> ) <sup>g</sup> (2580–4925)  3750 (LC <sub>50</sub> ) <sup>g</sup> (2580–4925)	Holdway (1992)
	Synthetic Magela Creek	6.0 ± 0.1	3.9 (3.8–4.0)	4.1 (4.0–4.2)	168 (+168 h post exposure)  96 h survival	3078 (LC <sub>50</sub> ) <sup>g</sup> (2580–3590)  1270 (BEC <sub>10</sub> ) <sup>b</sup> 1300 (MDEC) <sup>b</sup> 1570 (LC <sub>50</sub> ) <sup>g</sup> (1510–1630)	Markich & Camilleri (1997)

a. All numerical values represent mean values, or their range, with 95% confidence intervals (C.I.) in parentheses (where reported). Means shown with ± values were regulated within the reported limits. NR: not reported. Uranium (U) concentration is expressed as uranyl (ie UO<sub>2</sub>); this was derived by multiplying the U concentration by 1.14.

b. BEC<sub>10</sub>, 10% bounded-effect concentration (Hoekstra & van Ewijk 1993), an analogous statistical measure of the no-observed effect concentration (NOEC)

c. MDEC, minimal detectable concentration (Ahsanullah & Williams 1991), an analogous statistical measure of the lowest-observed effect concentration (LOEC)

d. EC<sub>50</sub>, median effect concentration

e. NOEC, no-observed effect concentration

f. LOEC, lowest-observed effect concentration

g. LC<sub>50</sub>, concentration at which there is 50% survival

#### *Purple-spotted gudgeon (M. mogurnda)*

Bywater et al (1991) compared the relative sensitivity of six fish species at various life stages to U in natural Magela Creek water over a 96 h period, to establish the most suitable species to assess the toxicity of U mine wastewater. *Mogurnda mogurnda* was found to be the third most sensitive species; being less sensitive than delicate blue-eye (*Pseudomugil tenellus*) and reticulated perchlet (*Ambassis macleayi*); and more sensitive than Mariana's hardyhead (*Craterocephalus marianae*), black-striped rainbowfish (*Melanotaenia nigrans*) and chequered rainbowfish (*Melanotaenia splendida*). Based on the sensitivity to U, any of these species would be suitable to assess U mine wastewater. However, *M. mogurnda* proved to be the most acceptable, as the larval stages can be easily fed and produced in numbers sufficient for laboratory bioassays.

The toxicity of U to *M. mogurnda* appears to be very similar in natural creek water and synthetic water, when a similar life stage is used (ie sac-fry and/or larvae) (Bywater et al 1991, Holdway 1992, Markich & Camilleri 1997). Such a result is interesting given the synthetic water lacks a DOC component, and DOC is considered an influential factor on metal toxicity. The sensitivity of *M. mogurnda* sac-fry to U in natural creek water has also been investigated over longer exposure (ie 14 d) periods (Holdway 1992). It was found that *M. mogurnda* sensitivity to U did not necessarily increase with increasing exposure time up to 14 d. Furthermore, *M. mogurnda* mortality was significantly ( $P \leq 0.05$ ) delayed when placed in 'clean' water (ie at natural U concentration) for 15 d after being exposed to U for 14 d.

#### **1.3.4 Mechanisms of U toxicity in water**

To prevent metal toxicosis, aquatic organisms integrate their excretion and storage processes to manage metal uptake. Some organisms are able to regulate the levels of a particular metal in their bodies independently of environmental concentrations, while others accumulate the metal in their bodies, detoxifying when necessary (Hyne et al 1993).

Freshwater hydra, such as *H. viridissima*, are particularly sensitive to metals as they lack metal-binding proteins that sequester and detoxify metals (Hyne et al 1993). The symbiotic algae hosted by *H. viridissima* help regulate exposure to elevated levels of a metal by accumulating the metal and, if necessary, shedding it from the host tissue (Hyne et al 1993). Uranium has been found to accumulate in nematocysts of hydra and inhibit the replacement of discharged nematocysts, resulting in feeding dysfunction and reduced population growth (Hyne et al 1993). The walls of the nematocyst capsules are collagenous in nature (Blanquet & Lenhoff 1966) and like many other collagens may have an affinity for U (Anselme et al 1990).

In higher animals, the mechanism of U toxicity may be attributed to changes in cellular membrane permeability due to the binding of uranyl ions to phosphate ligands and to the inhibition of cellular carbohydrate metabolism (Ellender et al 1992). The principal effect is the inactivation of phosphate-containing molecules and biological ligands such as ATPase (Ellender et al 1992). Refer to section 1.2.4 for other generic mechanisms already outlined for Cu (eg hardness, pH etc).

#### **1.3.5 Effect of physico-chemical parameters on U toxicity**

Increasing water hardness and alkalinity are typically considered to reduce the toxicity of U to freshwater organisms (Tarzwell & Henderson 1960, Poston et al 1984, Parkhurst et al 1984, Barata et al 1998). However, such studies failed to define the effects of true water hardness (ie Ca and/or Mg concentration) independently of alkalinity and pH. For example, Parkhurst et al (1984) described the 96 h LC<sub>50</sub> of U in hard water (208 mg CaCO<sub>3</sub> L<sup>-1</sup> hardness, 53 mg CaCO<sub>3</sub> L<sup>-1</sup> alkalinity, pH 7.5) to be approximately 4-fold greater than in soft water (35 mg CaCO<sub>3</sub> L<sup>-1</sup> hardness, 11 mg CaCO<sub>3</sub> L<sup>-1</sup> alkalinity, pH 6.7) for juvenile brook trout (*Salvelinus*

*fontinalis*). Parkhurst et al (1984) described the relationship between hardness and toxicity as a function of carbonate alkalinity, which was supported by Poston et al (1984) using *D. magna*.

Under constant water hardness and pH conditions, alkalinity has been found to attenuate the effects of U toxicity to a freshwater bivalve (*Velesunio angasi*) (Markich et al 1996). In support of this relationship, geochemical speciation modelling found U toxicity to be inversely proportional to the percentage of  $\text{UO}_2\text{CO}_3$  in solution, implying  $\text{UO}_2\text{CO}_3$  is not toxic. Several studies agree that uranyl complexes are less toxic than  $\text{UO}_2^{2+}$  (Nakajima et al 1979, Poston et al 1984, Greene et al 1986). Poston et al (1984) proposed that U toxicity is ameliorated due to an increase in the formation of uranyl carbonate complexes reducing the free hydrated uranyl ion ( $\text{UO}_2^{2+}$ ) concentration. Markich and Jeffree (1994) suggested U toxicity is reduced by  $\text{Ca}^{2+}$  and/or  $\text{Mg}^{2+}$  ions competing with  $\text{UO}_2^{2+}$  for binding and transport sites at the cell membrane, without directly altering U speciation in water. The effect of true water hardness on the toxicity and bioavailability of U to freshwater biota has yet to be described.

Few studies have examined the effects of pH on U toxicity. Those that have used different test organisms making it difficult to directly compare studies. An autonomous increase in pH over a range of 2.0 to 7.0 has been reported to reduce U toxicity (Nakajima et al 1979, Greene et al 1986, Markich et al 2000). For example, Markich et al (2000) found a decrease in pH from 6.0 to 5.0 to have a 5-fold increase in U toxicity to a freshwater bivalve, *Velesunio angasi*, in synthetic Magela Creek water. The enhancing effects of pH on the toxicity of U were supported by large changes in U speciation, as predicted by geochemical speciation modelling (Markich et al 1996). Nakajima et al (1979) and Greene et al (1986) suggest that low pH inhibited the binding of U to *Chlorella* sp by protonation of weak, basic binding sites on the algal surface. In contrast to the response of *V. angasi* to U (Markich et al 2000), Franklin et al (1998) observed that a decrease in pH from 6.5 to 5.7 had a 2-fold reduction in U toxicity to a freshwater alga, *Chlorella* sp in synthetic Magela Creek water. The notion that  $\text{H}^+$  in solution is able to elicit a protective effect is gathering support (Crist et al 1988, Schenck et al 1988, Parent & Campbell 1994). It has been proposed that the  $\text{H}^+$  concentration either directly affects metal uptake or indirectly affects the chemical speciation of the dissolved metal (Franklin et al 1998). Uncoupling these two factors is necessary to correctly understand U toxicity and bioavailability to freshwater biota.

Controversy surrounds the effect hydrophilic organic ligands exert on the toxicity and bioavailability of U in aquatic systems. Uranium toxicity was found to decrease in the presence of organic ligands (model fulvic acid), by complexing cationic uranyl species (eg  $\text{UO}_2^{2+}$  and  $\text{UO}_2\text{OH}^+$ ) (Yong & Macaskie 1995, Markich et al 1996). In contrast, the complexation of uranyl with oxalate ( $[\text{UO}_2(\text{Ox})_2]^{2-}$ ) was found to enhance U toxicity to a lichen, *Cladonia rangiferina* (Boileau et al 1985).

#### 1.4 Aim of study

The aim of this study was to separate the effects of true water hardness (Ca and Mg) and alkalinity (carbonate), at a constant pH, on the toxicity of Cu and U to *H. viridissima* (Green hydra, population growth) and *M. mogurnda* (purple-spotted gudgeon, sac-fry survival). This study also attempted to investigate the effects of pH (proton concentration), at constant hardness and alkalinity, on the toxicity of Cu and U to *H. viridissima* and *M. mogurnda* (see Riethmuller (2000), Appendix E for details). Gaining a fundamental understanding of how these parameters affect metal toxicity and bioavailability is an essential aspect of site-specific environmental risk assessment and water quality guideline derivation.